REMARKS

Status of the Claims

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30, and 31 are currently pending in the application. Claims 1 and 30 are amended without prejudice or disclaimer. Applicants reserve the right to claim the canceled subject matter in one or more divisional or continuation applications. As amended, claim 1 specifies "filtration *pressure*" in lieu of filtration depressure. Support for this element is found throughout the specification as originally filed including, *e.g.*, on page 19, lines 1-2. Claim 1 is further amended to specify that the maternal blood has been collected from a pregnant woman between the 5th and 15th week of pregnancy. Support for this element is found, *e.g.*, on page 7, lines 15 and 18. Claim 30 is amended to specify "1 X 10^5 pores/cm^2" in lieu of "1.10^5 pores/cm2." Support for this element is found, *e.g.*, on page 9, lines 14-15 in the originally filed application. No new matter has been added by way of the present amendments. Reconsideration is respectfully requested.

Issues Under 35 U.S.C. § 112, First Paragraph

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30, and 31 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement, *see* Office Action, page 2. Specifically, the Examiner states that the present application allegedly fails to support the concept of filtration pressure, *see* Office Action, page 3. In addition, the Examiner states that the present applications allegedly fails to support "a pore density of 1.10^5 pores/cm^2", *see* Office Action, page 3.

Although Applicants do not agree that the instant claims fail to comply with the written description requirement, in order to expedite prosecution, claim 1 is amended to specify "filtration pressure." In addition, claim 30 is amended to specify "1 X 10^5 pores/cm^2." Support for these amendments is described above. Claims 4, 5, 9, 11-18, 20-22, 25 and 31 are dependent on amended claim 1 and, accordingly, do not incorporate the allegedly unsupported phrase. Based upon the foregoing, Applicants submit that the rejection is overcome and respectfully request withdrawal of the rejection.

Issues Under 35 U.S.C. § 112, Second Paragraph

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30, and 31 are rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite, *see* Office Action, pages 3-4. Specifically, the Examiner states that the phrase "applied filtration depressure" is unclear, *see* Office Action, page 4.

Although Applicants do not agree that the claims lack clarity, claim 1 is amended in order to expedite prosecution to specify "filtration pressure." Dependent claims 4, 5, 9, 11-18, 10-22, 25, 30, and 31 incorporate the elements of amended claim 1. Based upon the foregoing, Applicants submit the claims are not indefinite and respectfully request the withdrawal of the rejection.

Issues Under 35 U.S.C. § 103(a)

Claims 1, 4, 5, 9, 11, 12, 20-22, 25, and 31 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Kalionis in view of Vona, further in view of Bisconte, *see* Office Action, pages 4-13.

Claims 13, 14, 16, and 17 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Kalionis in view of Vona and Bisconte, and further in view of Bianchi, *see* Office Action, pages 13-15.

Claim 15 is rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Kalionis in view of Vona and Bisconte, and further in view of Fodor, *see* Office Action, pages 15-16.

Claim 18 is rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Kalionis in view of Vona and Bisconte, and further in view of Pinkel, *see* Office Action, pages 16-17.

Claim 30 is rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Kalionis in view of Vona and Bisconte, and further in view of Whatman® Cyclopore® Membranes *see* Office Action, pages 17-19.

Claim 1, as amended, specifies that the maternal blood has been collected from a pregnant woman between the 5th and 15th week of pregnancy. This feature illustrates the performance of

the claimed invention, which enables the collection of fetal cells from maternal blood at a stage where they are very rare. Applicants submit that the Kalionis reference is silent with respect to this feature.

Furthermore, the method described in the Kalionis reference is enabled for pregnant women, who are at the end of gestation, *e.g.*, in their 30-37 weeks of gestation, *i.e.*, 7½ months of gestation and over, *see* Table 1 of the Kalionis reference, at page 21.

The other prior art references, including the Vona, Bisconte, Bianchi, Fodor, Pinkel and Whatman® Cyclopore® Membranes references, do not overcome this deficiency.

The method of Kalionis might be suitable for late stage gestation, but not for early stage gestation, in contrast to the presentluy claimed invention.

Indeed, the Kalionis method is not performed on an isolated fetal cell (nor on isolated fetal cells), but on a mixed population, which is enriched in fetal cells (trophoblast cells), but which still comprises other cell types (including maternal cells).

To the contrary, the method of instant claim 1 is enabled for pregnant women at any stage of pregnancy, and more particularly at the very early stages of pregnancy, where fetal cells are very rare, *e.g.*, as few as 1 one cell per mL, *i.e.*, one fetal cell per 10 million leukocytes (*cf.* page 27 lines 20-22 of the application as filed).

Furthermore, Applicants traverse these rejections as set forth in their Replies of November 28, 2006, of March 28, 2007, of October 31, 2007 and of August 5, 2008, the entireties of which are incorporated herein by reference as if each and every statement were represented herein, to address each and every rejection listed above.

More specifically, Applicants maintain that neither Kalionis nor Vona, nor any of the other references, disclose a step, which would resemble step b) of claim 1.

The method of Kalionis comprises:

- filtering a sample of the maternal blood,
- collecting all the cells that are retained on the filter, and then

- submitting all the collected cells to immunostaining with trophoblast-reactive antibodies, to identify the trophoblast cells, and then
- submitting the cells to in situ hybridization.

Contrary to step c) of the claimed invention, the Kalionis reference does not disclose and does not teach collecting one individual cell from the filter. In the Kalionis reference, there is no step, wherein a cell, which would be presumed of being of fetal origin, would be isolated.

Contrary to step c) of the claimed invention, the Kalionis reference does not disclose and does not teach a step, which comprises analyzing the cells that are retained on the filter before individually collecting an appropriate cell therefrom, *i.e.*, a cell, the (fetal) origin of which would be presumed.

Therefore, the Kalionis reference does not disclose a step that would resemble step c) of the claimed method.

Hence, both the nature and the order of the method steps of the Kalionis reference differ from those of claim 1.

It should also be duly taken into account that, in accordance with step f) of the claimed invention, the demonstration of fetal origin, and the prenatal diagnosis, are both performed on one single cell. More precisely, these steps are performed on the pre-amplified genome of one single trophoblastic and/or syncytiotrophoblastic cell

The Kalionis reference does not disclose and does not teach any step that resembles step f) of the claimed invention, *i.e.*, a step wherein a dual genetic analysis is performed (to confirm the fetal origin of the cell and to detect a genetic or chromosomic anomaly).

Hence, Applicants maintain that, starting from the Kalionis reference, and whichever document is combined therewith, one of ordinary skill in the art has to change everything in the Kalionis disclosure to arrive at the claimed invention.

Applicants also maintain that the Kalionis reference is not enabled to address the problem of pre-natal diagnosis.

In the instant Office Action, the document with which the Kalionis document is primarily combined is the Vona reference, which relates to a method of Isolation by Size of Epithelial Tumors cells (ISET method). In the Vona reference, there is only one single assay comprising both ISET and RT-PCR. This (ISET + RT-PCR) assay is performed on an epithelial tumor cell line, namely the Hep3B cell line.

In this assay, all the cells that are retained on the ISET filter are tumor cells, whereas, in the presently claimed method, the cells that are retained on the filter are a complex population of cells comprising a mixture of different cell types, such as fetal and maternal cells.

In the Vona reference, the starting cell population is a very simple one: it is a pure population of tumor cells from a cell line. Therefore, the Vona reference does not address the problem of identifying rare cells within a larger and complex cell population.

In contrast, the presently claimed invention solves the problem of the isolation of fetal circulating cells, *i.e.*, of cells that are *very rare* and that are contained in vivo in mixture with many other cell types: the starting material is a very complex population of many different types of cells, which include maternal epithelial cells and leukocytes.

As a matter of fact, the Vona reference does not disclose a step, which would comprise analyzing the cells that are retained on the filter before individually collecting an appropriate cell therefrom, *i.e.*, a cell, the (fetal) origin of which would be presumed.

Therefore, the Vona reference does not disclose and does not teach a step that would resemble step c) of the presently claimed method. Therefore, the Vona reference does not overcome the deficiency of the Kalionis reference in this respect.

Furthermore, in constrast to step f) of the claimed invention, the Vona reference does not disclose a method, wherein the amplification step would comprise the demonstration of two clinical features, whereas in the claimed method of the present invention, the amplification step is intended for both demonstrating the fetal origin of the collected cells and for carrying the prenatal diagnosis as such and both demonstrations are performed on the genome of one single cell (see step f) of the claimed method.

Therefore, the Vona reference does not overcome the deficiency of the Kalionis reference in this respect either.

Furthermore, whereas the Vona reference suggests testing whether ISET would be applicable to the filtration of trophoblast cells, it nevertheless does not suggest applying (ISET + RT-PCR) or (ISET + PCR), or more precisely (ISET + immunological/cytological analysis + PCR), to trophoblast cells. Therefore, the Vona reference does not disclose and does not suggest a method comprising both steps c) and f) of the claimed invention.

Once again, the Vona reference does not overcome the deficiency of the Kalionis reference in this respect.

In addition, none of the other cited references cited by the Examiner, including the Bisconte, Bianchi, Fodor and Pinkel references, overcome any of the various deficiencies of the Kalionis and/or the Vona reference(s) described above.

It is more particularly submitted that neither Kalionis nor Vona nor Bisconte, as well as the other cited references including the Bianchi, Fodor, Pinkel and Whatman® Cyclopore® Membranes, teach or suggest step c) of the claimed method.

It is more particularly submitted that neither Kalionis nor Vona nor Bisconte, nor the other cited references including the Bianchi, Fodor, Pinkel and Whatman® Cyclopore® Membranes references, teach or suggest step f) of the claimed method.

It is more particularly submitted that neither Kalionis nor Vona nor Bisconte, nor the other cited references, including the Bianchi, Fodor, Pinkel and Whatman® Cyclopore® Membranes references, teach or suggest step c) and step f) of the claimed method.

None of the cited references, more specifically Kalionis, Vona, Bisconte, Bianchi, Fodor and Pinkel references, disclose the filtration step of the claimed invention. Therefore, whichever reference combination is made, the person of ordinary skill in the art cannot arrive at the claimed invention.

It is more particularly submitted that, as previously mentioned, the claimed invention relates to a very specific problem, *i.e.*, the problem of pre-natal diagnosis, and that it involves

trophoblast cells, *i.e.*, cells that are very rare in the maternal blood. The claimed invention cannot be equated to any standard kind of cell analysis.

With the purpose of addressing the specific problem of the isolation of said very rare circulating fetal cells, in such a way as to enable a reliable pre-natal diagnosis, the inventor has determined which parameters had to be the most carefully chosen and at which most preferable value ranges these critical parameters should be adjusted.

These critical parameters notably include the choice of a filter with a particular pore density, and the application of a particular pressure range.

The Kalionis reference does not disclose nor suggest using a filter, which would have a pore size of 8 μm and a pore density in the range 5×10^4 to 5×10^5 pores/cm^2, and does not disclose or suggest applying any kind of filtration pressure. The Kalionis reference is silent about the pore density and pressure parameters.

Turning now to the Vona reference, when the reader has foreknowledge of the presently claimed invention, the reader can point out that the Vona reference discloses the use of "a polycarbonate Track-Etch-type membrane (Cyclotron Technology) with calibrated, 8-μm-diameter, cylindrical pores", and to apply "gentle aspiration under vacuum (created by a vaccum pump)." However, it must, at the very least, be acknowledged that the Vona reference does not disclose a $5x10^4$ to $5x10^5$ pore density range, and does not disclose a 0.05-0.8 bar range.

Most importantly, it is only when the reader has foreknowledge of the claimed invention that these two passages of the Vona reference can be selected among all the other parameters disclosed in said reference.

When the Vona reference is read without foreknowledge of the claimed invention, it should be recognized that this reference contains no indication, which would specifically direct the person of ordinary skill in the art toward the presently claimed pore density and pressure parameters rather than toward any other of the various parameters of the method disclosed in the Vona reference.

Furthermore, as the person of ordinary skill in the art is arguably taking the Kalionis reference into account, this person would not have recognized the criticality of the pore density of the filter and not have associateed any criticality with the pressure (if any) that is applied for filtration, because the Kalionis reference, which arguably may disclose a pre-natal diagnosis method, is completely silent with respect to these two parameters. Therefore, these two parameters appear to be of no importance at all to the person of ordinary skill in the art.

As a matter of fact, there is no well-founded reason for the person of average skill in the art to select the pore density parameter and the pressure parameter among the various other potential parameters, of the method disclosed in the prior art references, more particularly in the Vona reference.

Asserting that the person of ordinary skill in the art would *e.g.*, somehow "adjust" these two parameters, when there are many other parameters that could potentially be "adjusted", would amount to an *ex post facto* reconstruction of the prior art references, more particularly of the Vona reference.

Moreover, at least at the time when the invention was made by the inventor, for each filter with a given pore diameter, very different pore density ranges were available, including pore densities that were outside of the $5x10^4$ to $5x10^5$ range. Therefore, a filter with a calibrated, 8 μm-diameter, cylindrical pores (as it is disclosed in the Vona reference) does not necessarily have a pore density of $5x10^4$ to $5x10^5$ pores/cm^2.

Furthermore, the Vona reference does not disclose and does not suggest the criticality of selecting a filter, wherein the pores are spaced apart to allow the separation and collection of individual cells.

If the person of ordinary skill in the art were trying to use the Vona method, which in the Vona reference is disclosed to be adapted to the isolation of tumor cells from a pure culture of tumor cells, and to apply it to achieve another and very different result, *i.e.*, the isolation of the very rare circulating fetal cells, the ordinary artisan would have no indication or direction anywhere in the cited references as to the solution of somehow "adjusting" the filter pore density and the filtration pressure value.

The other prior art references, including the Bisconte, Bianchi, Fodor, Pinkel and Whatman® Cyclopore® Membranes references, do not overcome the deficiencies of the Kalionis and/or Vona reference(s) as described.

It is therefore submitted that whichever combination is made of the cited references, many technical features, including steps b), c), and f) of the claimed invention, remain undisclosed and are not even suggested.

Thus, it is respectfully submitted that the Examiner has failed to establish a *prima facie* case of obviousness and that the claims satisfy the requirements of 35 U.S.C. § 103(a).

Furthermore, the amendments of the claims presented herein are believed to adequately address all of the Examiner's comments presented in the Office Action of October 15, 2008. That is, it is believed that the references, either considered individually or in combination, do not disclose or suggest all of the limitations recited in the presently amended claims. Thus, the Examiner has failed to establish a *prima facie* case of obviousness with respect to the instant claims.

Reconsideration and withdrawal of the obviousness rejections are respectfully requested.

CONCLUSION

In view of the above amendment and remarks, Applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Ph.D., Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By Linda T. Parker Reg. # 46046

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